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The Effects of Strenuous Exercise on Infection with *Francisella tularensis* in Rats

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To investigate the effects of strenuous forced exercise on the course and complications of a bacterial infection and on myocardial responses and performance capacity, rats with tularemia (characterized by pyogranulomatous hepatic and splenic lesions) were exercised by swimming on days 0-6 of infection. Levels of glutamic oxaloacetic and pyruvic transaminases in plasma, densities of pyogranulomatous lesions, and bacterial counts in blood, liver, and spleen were similar in exercising and resting rats. Although a few exercising rats showed an unusual dissemination of infection, the antibody responses were similar in rest and exercise. Plasma concentrations of β-glucuronidase, lysozyme, and α_2-macrofetoprotein were higher with exercise, a result that indicated that more vigorous stress responses were elicited with exercise than with infection alone. Physical performance capacity was reduced by the infection, but forced daily exercise limited this reduction substantially and counteracted the myocardial protein-degrading effects of infection. Thus, exercise evoked normal training responses even during this generalized infection.

There is convincing clinical and experimental evidence that physical activity may be harmful or may provoke complications in some infectious diseases in which the infectious process is localized in structures specifically activated by physical exercise, such as muscle or nerve tissue. For example, paralysis due to poliomyelitis is more extensive in exercising subjects [1, 2], and exercise-related increases in virus replication and tissue damage have been documented in coxsackievirus myocarditis [3].

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In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The animal facilities at Fort Detrick are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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These results cannot be extrapolated to infectious diseases in general because the invading microorganisms were causing direct damage to the physically active tissues. There are very few reports on whether physical exercise may be detrimental in the acute phase of a generalized infection. In viral hepatitis, rather strenuous physical exercise in early convalescence has been shown not to cause relapse or to prolong recovery time [4], and light exercise—such as ambulation on the ward—does not seem to be harmful even in the acute phase [5, 6].

Materials and Methods

Animals. Male Sprague-Dawley rats were used (Taconic Farms, Germantown, N.Y.). The rats were maintained on a commercial diet (Wayne LabBlox®; Allied Mills, Chicago) and housed in rooms maintained at 23 ± 1 C. Rats in study 1 were randomly divided into four groups: group A, infection plus exercise; group B, infection plus rest; group C, no infection plus exercise; and group D, no infection plus rest. Within each group, rats were preassigned to take part in the experiment for a total of two, four, or seven days. Rats were assigned to groups in numbers large enough to allow for losses due to lethality, which

was estimated for each group from preliminary experiments.

In study 2 additional rats were randomly placed in four similar categories for an additional exhaustion performance test on day 3. In study 3 the program of study 1 was repeated, but only infected rats (groups A and B) were used to test the pathologic and bacteriologic effects of exercise in various organs on day 3 after infection. The initial mean body weight for the groups of rats varied between 276 and 369 g.

Infection. On day 0 of each study, rats in groups A and B were inoculated ip with a 1-ml suspension of 3.65×10^7 cfu of unwashed *Francisella tularensis* (live vaccine strain)/ml; the organisms had been grown on solid fortified glucose-cysteine-blood agar [7]. Rats in groups C and D (noninfected rats) of studies 1 and 2 were inoculated with 1 ml of sterile tryptose saline (Difco Laboratories, Detroit). Although this strain of *F. tularensis* is attenuated in comparison with wild strains, the dose and route of administration have previously been shown to produce about 15% lethality in nonexercised Sprague-Dawley rats. In all three studies body temperatures were recorded daily before exercise and before blood and tissue sampling by a rectally inserted thermocouple.

Food and water were supplied ad libitum. The food was weighed daily before and after feeding, and the average individual food consumption was calculated for each group of rats.

Exercise. Rats were exercised by being forced to swim in steel barrels that were 50 cm in diameter and filled to a depth of 55 cm with water at 33-35 C. Ten rats were exercised in each barrel; this made it impossible for the animals to float quietly and rest because they were continually treading on their neighbors. To adapt the rats to the exercise situation, they were exercised for 10 min on day 0. On days 1-6, exercise was continued for a total of 3 hr per day, with a rest of 1 hr between the second and third hours. This exercise time was found to cause considerable exhaustion of the rats but led to very few drowning deaths. As the rats grew tired, they developed a characteristic behavior pattern in the water: with increasing frequency they touched the bottom to rest momentarily and then returned to the surface and continued swimming.

In study 2 performance capacity was estimated for each group of rats by measuring the swimming time for each rat on day 3 until individual exhaustion. The time at which the rat was unable to return to the surface after having touched the bottom was taken as the point of exhaustion [8]. Then an experienced technician, who closely supervised the exercise at all times, rescued the rat.

Sampling. On days 2, 4, and 7 in study 1 and on day 3 in study 3, eight to 10 preassigned rats from each group were anesthetized with halothane. Using sterile technique the thoracic cavity was opened, the caval veins were severed, and blood was collected from the right pleural cavity using pipettes treated with heparin (10 units/ml). Then the heart, liver, and spleen were removed under sterile conditions and weighed. In study 1, tissue from the ventricular myocardium was minced with scissors and homogenized in 20 volumes (wt/vol) of ice-cold 0.15 M KCl, 6 mM EDTA, and 40 mM $KHCO_3$ (pH 7.4) using manual all-glass homogenizers. The entire procedure was performed at 0-4 C. In study 3, half of each organ was placed in 10% buffered formalin for routine histologic processing and preparation of slides stained with hematoxylin and eosin. The other half of each organ was homogenized and plated in 10-fold serial dilutions on glucose-cysteine-blood agar plates. Blood was plated similarly.

Assays. Plasma. Blood plasma from rats was used in individual analyses of levels of zinc [9], β-glucuronidase (EC 3.2.1.31) [10], ornithine-carbamoyl transferase [11], lysozyme [12], antibodies to *F. tularensis* [13], and α_2-macrofetoprotein [14] (study 1). Furthermore, levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase were determined by standard methods [15, 16] (study 3).

Tissues. In study 1 homogenates were immediately frozen; on a later day they were thawed for analysis of activities of β-glucuronidase [17] and cathepsin D (EC 3.4.23.5) [10] and contents of protein [18], RNA, and DNA [19]. *F. tularensis* colonies in plated homogenates of myocardium, of liver and spleen, and of blood were counted after incubation for 48-72 hr at 37 C (study 3); histopathologic examinations of each of these tissues, except for blood, were evaluated, and the densities of pyogranulomatous lesions were graded on a scale of 0-4. The presence of *F. tularensis* antigens in dermal tissue was demonstrated by an immunochemiluminescence technique [20].

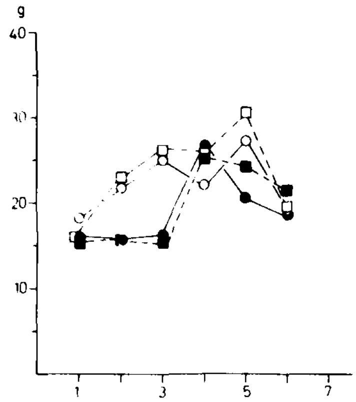

Figure 1. Effects in rats of infection with *Francisella tularensis* and of exercise on the average daily intake of food per animal. The rats were divided into group A (infection plus exercise [■]), group B (infection plus rest [●]), group C (no infection plus exercise [□]), and group D (no infection plus rest [O]).

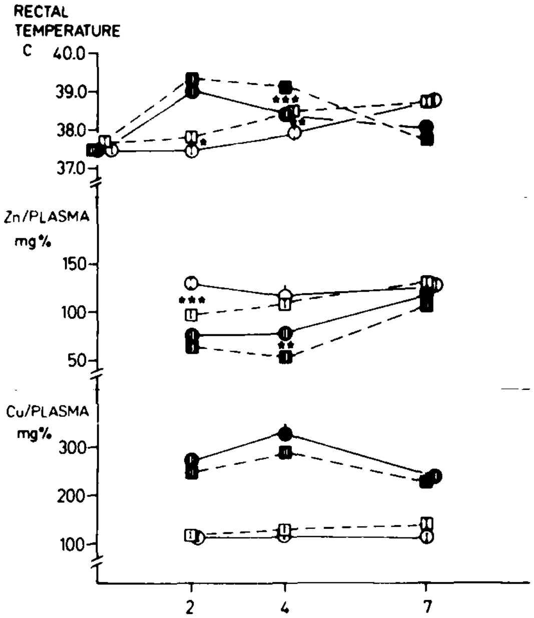

Figure 2. Effects in rats of infection with *Francisella tularensis* and of exercise on rectal temperatures and concentrations of zinc and copper in plasma at various times after inoculation. The rats were divided into group A (infection plus exercise [■]), group B (infection plus rest [●]), group C (no infection plus exercise [□]), and group D (no infection plus rest [O]). Values are means ± SE. Stars denote statistically significant differences between exercising and resting rats: (★) = $P < 0.05$; (★★) = $P < 0.01$; (★★★) = $P < 0.001$.

Statistics. For studies 1 and 2, the interacting effects of infection and exercise were calculated and evaluated for significance by a two-way analysis of variance. For all three studies, the differences between exercising and resting rats were tested further by Student's *t*-test. (Results of the two-way analysis of variance are included in the text and those of Student's *t*-test in the tables and figures.)

Results

Clinical observations and performance. Infected rats consumed less food than noninfected rats during the first three days of infection, but exercise had no discernible effects on the intake of food (figure 1).

Infection and exercise each had an augmenting effect on body temperature in the acute phase of infection (days 2 and 4) ($P < 0.001$ for each on day 4), whereas during the early convalescent period (day 7) infected rats had lower body temperatures than control animals ($P < 0.001$ on day 7) (figure 2). In two rats of group A (infection plus exercise), a few papulopustules were observed in the skin of

Table 1. Effects of prior exercise on the performance of infected and control rats exercised to exhaustion on day 3 after infection with *Francisella tularensis*.

Group	Prior exercise	Infection	Pre-exercise temperature (C)	Exercise time to exhaustion (min)	Significance
A	Yes	Yes	39.7 ± 0.2	268 ± 30	$P < 0.001$*
B	No	Yes	39.7 ± 0.2	153 ± 11	
C	Yes	No	38.6 ± 0.2	369 ± 26	NS†
D	No	No	38.3 ± 0.1	409 ± 32	

NOTE. Values are means ± SE.
* By Student's *t*-test.
† NS = not significant.

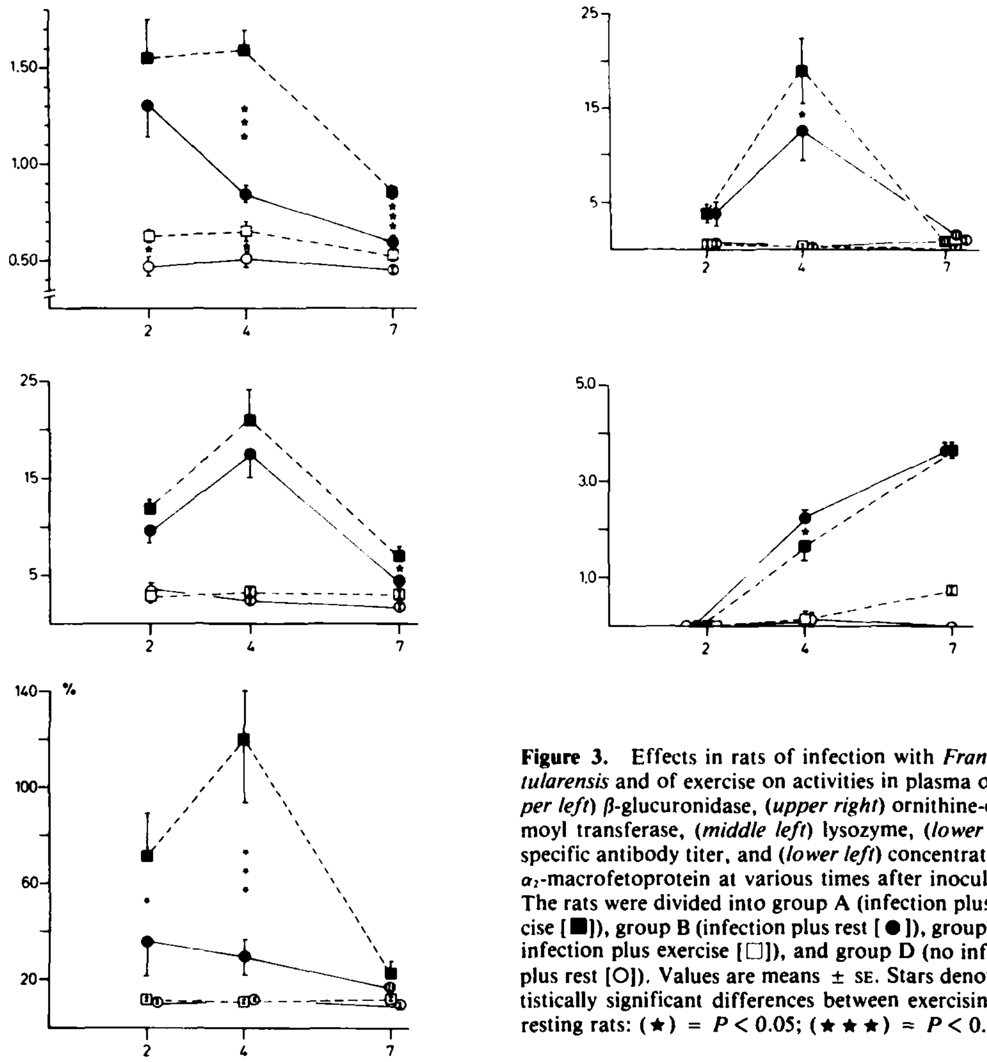

Figure 3. Effects in rats of infection with *Francisella tularensis* and of exercise on activities in plasma of (*upper left*) β-glucuronidase, (*upper right*) ornithine-carbamoyl transferase, (*middle left*) lysozyme, (*lower right*) specific antibody titer, and (*lower left*) concentration of α_2-macrofetoprotein at various times after inoculation. The rats were divided into group A (infection plus exercise [■]), group B (infection plus rest [●]), group C (no infection plus exercise [□]), and group D (no infection plus rest [○]). Values are means ± SE. Stars denote statistically significant differences between exercising and resting rats: (★) = $P < 0.05$; (★★★) = $P < 0.001$.

the thorax or upper abdomen. The papulopustules were biopsied, and their pustular nature was established. Furthermore, *F. tularensis* antigen was detected in these lesions [20]. No papulopustules were found in rats of any of the other groups; such dermal lesions were not observed in our preliminary studies with this dose or strain of *F. tularensis*.

Performance capacity on day 3 of the infection was reduced to 37.4% of control values in those rats that were allowed to rest during days 0–3 (groups B and D), whereas in previously exercised rats (groups A and C) the corresponding figure was 72.6%. This infection-related reduction in performance was highly significant ($P < 0.001$). The three-day exercise program improved the performance capacity of the infected but not the noninfected rats (table 1).

During the exercise sessions, a few infected rats died suddenly and unexpectedly. Without warning, these rats sank to the bottom and made no attempt to come up again. Sudden death occurred at various times during a session and did not occur in noninfected rats. In general, infected rats became exhausted more suddenly than noninfected ones. They usually exercised vigorously until that point.

Plasma analyses. The decrease in the plasma level of zinc paralleled the fever reaction in response to infection and exercise during the acute phase of infection (days 2 and 4) ($P < 0.001$ for infection; $P < 0.001$–0.01 for exercise), whereas an increase in the plasma level of copper occurred only with infection ($P < 0.001$) (figure 2).

Plasma levels of β-glucuronidase were significantly ($P < 0.001$) elevated by both infection and exercise, but the effect of exercise was more pronounced in the infected rats (figure 3, upper left). Apart from a somewhat more pronounced elevation in the levels of ornithine-carbamoyl transferase on day 4 of infection (figure 3, upper right), exercise did not increase activities of liver enzymes in plasma (figure 4). The concentration of lysozyme was slightly higher in the infected rats that exercised than in their resting counterparts (figure 3, middle left). The antibody response to *F. tularensis* antigen as a result of the infection reached the same titer by day 7 in both exercising and resting rats (figure 3, lower right). α_2-Macrofetoprotein appeared in plasma only in infected rats, and exercise alone did not cause an elevation. However, the concentration was up to four times higher in those infected rats that exercised (figure 3, lower left).

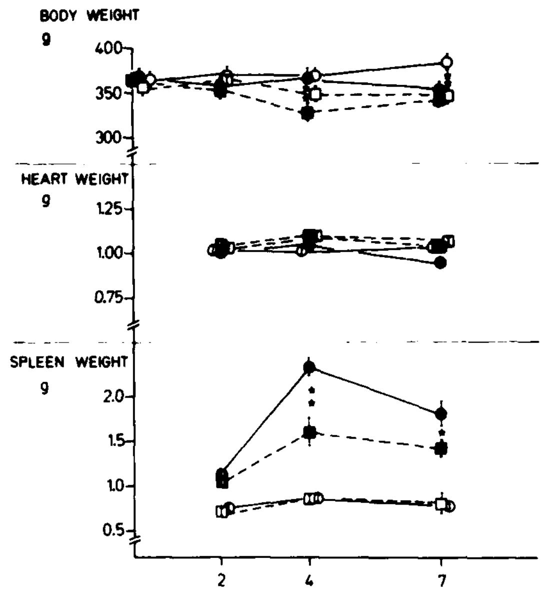

Figure 5. Effects in rats of infection with *Francisella tularensis* and of exercise on body, heart, and spleen weights at various times after inoculation. The rats were divided into group A (infection plus exercise [■]), group B (infection plus rest [●]), group C (no infection plus exercise [□]), and group D (no infection plus rest [○]). Values are means ± SE. Stars denote statistically significant differences between exercising and resting rats: (★) = $P < 0.05$; (★★) = $P < 0.01$.

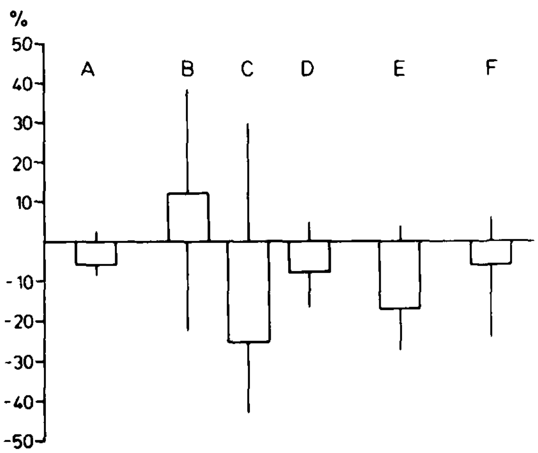

Figure 4. Effects of exercise in rats infected with *Francisella tularensis* on measures of pathology of the liver. Means ± SE are shown for liver weight (A), activity in plasma of glutamic oxaloacetic transaminase (B), activity in plasma of glutamic pyruvic transaminase (C), activity in plasma of alkaline phosphatase (D), density of pyogranulomatous lesions (E), and *F. tularensis* count per gram of tissue (F). No differences were significant.

Tissue analyses. Body weight was decreased by exercise on days 4 and 7 ($P < 0.01$), whereas infection caused a smaller loss ($P < 0.05$ on day 7) (figure 5). Heart weight was not influenced by either infection or exercise; the hearts of animals in all of the groups showed normal histologic results with no signs of myocarditis, and cultures of heart tissue showed no bacteria. In the liver, pyogranulomatous lesions—which are characteristic of infection with *F. tularensis* in rats [16]—were of similar density in exercising and in resting infected rats; similar amounts of *F. tularensis* were grown from each gram of liver tissue in these two groups (means ± SE, 5.02 ± 0.98 and 5.34 ± 0.74 log/g of tissue, respectively), and the liver weight was the same (figure 4). The infection caused the spleen to increase in size ($P < 0.001$ on all days); the weight was tripled in resting rats on day 4. Spleen enlargement was significantly less pronounced in exercising rats (figure 5). However, the density of pyogranulomatous lesions and the bacterial counts per gram of tissue were not influenced by exercise and were of the same order of magnitude as in the

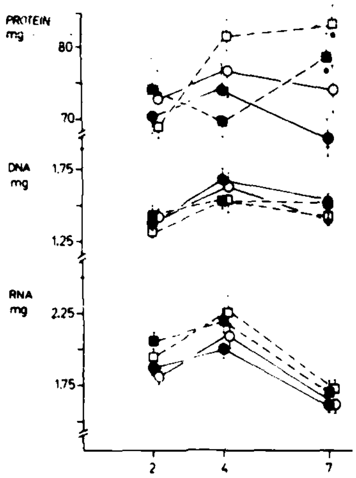

Figure 6. Effects in rats of infection with *Francisella tularensis* and of exercise on the total content of protein, DNA, and RNA in heart muscle at various times after inoculation. The rats were divided into group A (infection plus exercise [■]), group B (infection plus rest [●]), group C (no infection plus exercise [□]), and group D (no infection plus rest [O]). Values are means ± SE. (★) = $P < 0.05$.

liver. Blood cultures showed similar amounts of *F. tularensis* in exercising and resting infected rats (means ± SE, 1.57 ± 0.40 and 1.66 ± 0.41 log/ml, respectively).

The total myocardial content of protein was significantly reduced on days 4 and 7 ($P < 0.05$) as an effect of the infection, but simultaneously the exercise had a protein-stimulating effect ($P < 0.01$ on day 7). The exercise-induced increase was of the same magnitude in infected as in noninfected rats (figure 6). The content of DNA or RNA in the heart was not significantly influenced by either infection or exercise (figure 6).

The activity of β-glucuronidase in the heart was elevated as a result of infection ($P < 0.001$ on day 7), whereas activation induced by exercise was less evident ($P < 0.05$ on day 7) and of similar magnitudes in infected and noninfected rats. Cathepsin D showed an essentially similar pattern with some activation induced by infection ($P < 0.05$ on day 4) and some by exercise ($P < 0.05$ on day 7) (figure 7).

Discussion

Forced daily swimming exercise during acute infection with *F. tularensis* in rats did not alter the overall progression and severity of the disease, although occasionally signs of dissemination of the infection occurred. For previously sedentary rats, the capacity for exercise was reduced by the infection to almost one third of that of control animals, but the maintenance of a brief daily exercise schedule limited this reduction substantially during the acute phase of illness. The response of the myocardium to the training stimulus of daily exercise was not altered by infection with *F. tularensis*. Myocarditis did not occur. The increases in the activity of lysosomal enzymes and in levels of protein in the myocardium that were produced by exercise were of similar magnitude in infected and noninfected animals.

Not unexpectedly, tularemia produced an increased body temperature, a transient decrease in dietary intake, a decrease in plasma levels of zinc, and an increase in plasma levels of copper [7, 9]. The initial days of exercise also caused body temperatures to rise, although the rats had been resting for 18–20 hr before each temperature measurement. The increase in temperature may indicate an increased metabolic turnover rate in exercising rats before they had become adapted to the exercise because no difference in temperature was evident on day 7 (figure 2). The reduced plasma concentrations of zinc in exercising rats cannot be explained by an inflammatory response alone be-

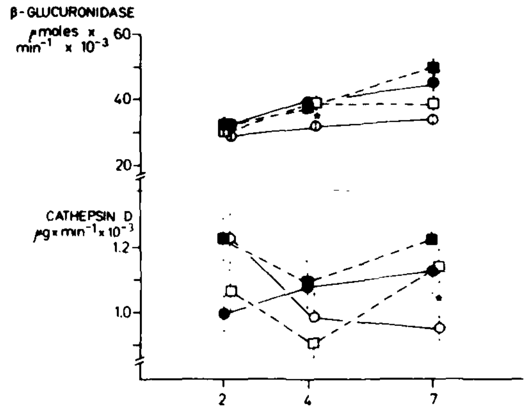

Figure 7. Effects in rats of infection with *Francisella tularensis* and of exercise on the total activity of β-glucuronidase and cathepsin D in heart muscle at various times after inoculation. The rats were divided into group A (infection plus exercise [■]), group B (infection plus rest [●]), group C (no infection plus exercise [□]), and group D (no infection plus rest [O]). Values are means ± SE. (★) = $P < 0.05$.

cause increases in levels of α_2-macrofetoprotein did not occur as a result of exercise.

The presence of skin pustules that contained *F. tularensis* at a site distant from the inoculation in two exercising rats suggested a particularly long-lasting bacteremia in combination with lowered local tissue defense mechanisms. Blood cultures showed similar concentrations of *F. tularensis* in exercising and resting rats. The skin pustules may have been provoked by exercise.

The different behavior of infected compared with noninfected rats during exercise is noteworthy. The former seemed to become exhausted more rapidly, whereas the latter reached exhaustion more gradually. Some infected rats, but none of the noninfected group, stopped swimming suddenly during the early stages of exercise for reasons that are obscure. Possibly sudden-death syndrome was more readily elicited in rats that were exposed to the stresses of both exercise and infection. Congestive heart failure and death during swimming exercise in the acute phase of myocarditis due to coxsackievirus B3 in mice were explained by an increased replication of virus in the myocardium [3]. In the present studies the lack of change in heart weights, the normal histologic results, and the negative results of cultures of myocardial tissue rule out development of clinical or subclinical myocarditis, as defined in pathologic and anatomic terms [3]. Sudden death is a previously recognized phenomenon that may occur in apparently healthy rats under conditions of heavy stress, including swimming exercise [21]. Although we did not observe such deaths in our control groups in the present study, we have in other work occasionally encountered the phenomenon. The added stress of infection may have increased the likelihood of sudden death during exercise. The mechanism of sudden-death syndrome remains unexplained but may be associated with increased parasympathetic stimulation followed by cardiac arrest [21]; the incidence of the syndrome in normal swimming rats has also been increased by trimming off the whiskers, thereby depriving the rat of important sensory input [21].

In biochemical terms, the heart responded to exercise in a similar fashion in infected and noninfected rats. Thus, the protein content in the myocardium increased in similar amounts after one week of daily exercise (figure 6) as a normal response to training [22]. Furthermore, the exercise program caused activation of the lysosomal enzymes, β-glucuronidase and cathepsin D, to a similar magnitude in both sick and healthy rats. The magnitude of the increases in enzyme activity in the myocardium of 8%-17% on day 7 (figure 7) is compatible with a normal training response when compared with findings reported for skeletal muscle of mice [23]. Similarly, the increase in enzyme activity in the myocardium induced by tularemia was comparable to that found in skeletal muscle [24].

An important finding in the present study involves the similar myocardial response in terms of increases in levels of protein and in the activity of lysosomal enzymes in infected and noninfected rats after similar amounts of exercise. Thus, the anabolic stimulus of exercise training and the catabolic one of infection seem to elicit their responses independently. The fact that the relative exercise load was higher in the sick rats because of their reduced performance capacity does not invalidate this conclusion.

A reduction of the amount of protein in the myocardium as a result of Newcastle disease in young chicks was accompanied by decreases in amounts of DNA and RNA and in heart weight [25]. In the present study, only the protein content was reduced, whereas the content of DNA and RNA and the heart weight were not influenced by the infection. A comparative study of tularemia and influenza with similar lethality in mice showed similar differences, a result which suggests that viral infections are more detrimental to the heart than bacterial infections [26].

The more pronounced elevation of plasma β-glucuronidase and ornithine-carbamoyl transferase in exercising than in resting rats during tularemia suggests an increased release of enzymes from the liver; in tularemia of rats, most plasma β-glucuronidase is believed to emanate from this organ [27], and ornithine-carbamoyl transferase is virtually liver-specific [11]. Even muscle contains β-glucuronidase at a low concentration [28], and release from muscle may explain the moderate elevation even in noninfected exercising rats (figure 3). The somewhat higher levels of plasma lysozyme with exercise than with rest during infection may also favor a more profound tissue involvement in exercise; in tularemia of rats, levels of plasma lysozyme correlate with the density of pyogranulomatous lesions in the liver, which are characteristic of this infection [27]. However, plasma levels of glutamic oxaloacetic transaminase, glu-

tamic pyruvic transaminase, and alkaline phosphatase were similar in resting and exercising rats, and the severity of histologic lesions in the liver and spleen and the bacterial concentration in these organs were not changed by the exercise program.

α_2-Macrofetoprotein is an acute-phase reactant in rats. Concentrations have been shown to increase during infection with *F. tularensis* to levels similar to those found in our nonexercising rats [15]. The stress of exercise did not elicit any response in noninfected rats, but the concentration was up to four times higher in those infected rats that exercised (figure 3, lower left). This observation supports the concept that the inflammatory process was more severe in exercising rats than in resting rats. The results cannot be explained as merely the addition of the responses to the stresses of infection and exercise.

Spleen weight usually correlates with the severity of tularemia in rats [16]. The reason for the lower spleen weight in our exercising infected rats compared with infected control animals is not clear. No histologic abnormalities other than pyogranulomatous lesions were observed.

A trend was observed for specific antibodies to respond more slowly in the exercising rats (figure 3, lower right), but after seven days similar titers were reached in both exercising and resting rats. Reyes and Lerner [29] found that exercise in mice infected with coxsackievirus B3 was accompanied by reduced titers of specific antibody in serum.

References

1. Horstmann, D. M. Acute poliomyelitis: relation of physical activity at the time of onset to the course of the disease. J.A.M.A. 142:236-241, 1950.
2. Rosenbaum, H. E., Harford, C. G. Effect of fatigue on susceptibility of mice to poliomyelitis. Proc. Soc. Exp. Biol. Med. 83:678-681, 1953.
3. Lerner, A. M., Wilson, F. M. Virus myocardiopathy. Prog. Med. Virol. 15:63-91, 1973.
4. Edlund, A. The effect of defined physical exercise in the early convalescence of viral hepatitis. Scand. J. Infect. Dis. 3:189-196, 1971.
5. Chalmers, T. C., Eckhardt, R. D., Reynolds, W. E., Cigarroa, J. G., Jr., Deane, N., Reifenstein, R. W., Smith, C. W., Davidson, C. S. The treatment of acute infectious hepatitis: controlled studies of the effects of diet, rest, and physical reconditioning on the acute course of the disease and on the incidence of relapses and residual abnormalities. J. Clin. Invest. 34: 1163-1235, 1955.
6. Nefzger, M. D., Chalmers, T. C. The treatment of acute infectious hepatitis: ten-year follow-up study of the effects of diet and rest. Am. J. Med. 35:299-309, 1963.
7. Neufeld, H. A., Pace, J. A., White, F. E. The effect of bacterial infections on ketone concentrations in rat liver and blood and on free fatty acid concentrations in rat blood. Metabolism 25:877-884, 1976.
8. Foss, C. R., Horvath, S. M. Reactions of wild and albino mice in response to forced swimming. Proc. Soc. Exp. Biol. Med. 120:588-592, 1965.
9. Pekarek, R. S., Burghen, G. A., Bartelloni, P. J., Calia, F. M., Bostian, K. A., Beisel, W. R. The effect of live attenuated Venezuelan equine encephalomyelitis virus vaccine on serum iron, zinc, and copper concentrations in man. J. Lab. Clin. Med. 76:293-303, 1970.
10. Canonico, P. G., Bird, J. W. C. Lysosomes in skeletal muscle tissue: zonal centrifugation evidence for multiple cellular sources. J. Cell Biol. 45:321-333, 1970.
11. Ceriotti, G. Ornithine carbamoyltransferase. *In* H. U. Bergmeyer [ed.]. Methods of enzymatic analysis. Vol. 2. 2nd ed. Academic Press, New York, 1974, p. 691-698.
12. Osserman, E. F., Lawlor, D. P. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. J. Exp. Med. 124:921-952, 1966.
13. Massey, E. D., Mangiafico, J. A. Microagglutination test for detecting and measuring serum agglutinins of *Francisella tularensis*. Applied Microbiology 27:25-27, 1974.
14. Weimer, H. E., Benjamin, D. C. Immunochemical detection of an acute-phase protein in rat serum. Am. J. Physiol. 209:736-744, 1965.
15. Powanda, M. C., Cockerell, G. L., Moe, J. B., Abeles, F. B., Pekarek, R. S., Canonico, P. G. Induced metabolic sequelae of tularemia in the rat: correlation with tissue damage. Am. J. Physiol. 229:479-483, 1975.
16. Moe, J. B., Canonico, P. G., Stookey, J. L., Powanda, M. C., Cockerell, G. L. Pathogenesis of tularemia in immune and nonimmune rats. Am. J. Vet. Res. 36:1505-1510, 1975.
17. Barrett, A. J. Lysosomal enzymes. *In* J. T. Dingle [ed.]. Lysosomes: a laboratory handbook. North-Holland, Amsterdam, 1972, p. 46-135.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.
19. Wannemacher, R. W., Jr., Banks, W. L., Jr., Wunner, W. H. Use of a single tissue extract to determine cellular protein and nucleic acid concentrations and rate of amino acid incorporation. Anal. Biochem. 11:320-326, 1965.
20. Reichard, D. W., Miller, R. J., Jr. Chemiluminescence immunoreactive assay (CLIA): a rapid method for the detection of bacterial and viral agents—*Francisella tularensis*, live vaccine strain (LVS) and Venezuelan equine encephalomyelitis vaccine strain (VEE TC-83). Army Science Conference Proceedings 3:169-179, 1980.
21. Richter, C. P. On the phenomenon of sudden death in animals and man. Psychosom. Med. 19:191-198, 1957.
22. Holloszy, J. O., Booth, F. W. Biochemical adaptations to endurance exercise in muscle. Annu. Rev. Physiol. 38: 273-291, 1976.
23. Vihko, V., Salminen, A., Rantamäki, J. Exhaustive exercise, endurance training, and acid hydrolase activity in skeletal muscle. J. Appl. Physiol. 47:43-50, 1979.

24. Friman, G., Ilbäck, N.-G. Effects of bacterial infections on oxidative and glycolytic enzyme activity in red and white skeletal muscle of the rat [abstract]. Clinical Research 28:643A, 1980.
25. Squibb, R. L., Lyons, M. M., Beisel, W. R. Virus involvement in the avian heart: effect on protein synthesis. J. Nutr. 96:509-512, 1968.
26. Ilbäck, N.-G., Friman, G., Beisel, W. R. Catabolic alterations in the mouse heart caused by *Francisella tularensis* and influenza infection [abstract]. Clinical Research, 1982 (in press).
27. Canonico, P. G., Powanda, M. C., Cockerell, G. L., Moe, J. B. Relationship of serum β-glucuronidase and lysozyme to pathogenesis of tularemia in immune and non-immune rats. Infec. Immun. 12:42-47, 1975.
28. Bird, J. W. C. Skeletal muscle lysosomes. *In* J. T. Dingle and R. T. Dean [ed.]. Lysosomes in biology and pathology. Vol. 4. North-Holland, Amsterdam, 1975, p. 75-109.
29. Reyes, M. P., Lerner, A. M. Interferon and neutralizing antibody in sera of exercised mice with coxsackievirus B-3 myocarditis. Proc. Soc. Exp. Biol. Med. 151: 333-338, 1976.